

OBSERVATIONS ON THE GIANT AMOEBA, AMOEBA CAROLINENSIS (WILSON, 1900)¹

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INTRODUCTION

During the past few years there has been considerable controversy over the name of the giant fresh-water amoeba which Wilson (1900) described and named *Pelomyxa carolinensis*. He placed this amoeba in the genus *Pelomyxa* evidently because it has many nuclei.

Mast (1938) and Rice (1945) agree with Wilson in regard to the name of this form. The latter author says that the principal characteristic of the genus *Pelomyxa* is its many nuclei. Greeff (1874), who established the genus, states that besides vacuoles there are in *Pelomyxa palustris* three kinds of characteristic structures: (1) Nuclei, (2) Hyaline and homogeneous bodies of spherical, ellipsoidal, or irregular shape, and (3) *Stäbchen*, little rods (later considered to be symbiotic bacteria). He does say that the large number of nuclei forms a principal characteristic of the genus *Pelomyxa*. However, since Greeff's description of *P. palustris*, pelomyxae have been described with one or few nuclei (*P. Belevskii* Penard, 1888; *P. binucleata* (Gruber, 1885) Penard, 1902; *P. paradoxa* Penard, 1902; *P. lentissima* Schaeffer, 1918; *P. schiedti* Schaeffer, 1918), and amoeboid forms other than pelomyxae have been described with more than one nucleus. *Amoeba proteus* has been observed in this laboratory with as many as eight nuclei.² Also the hyaline spherical bodies are not found exclusively in *Pelomyxa*. In view of these facts, Penard (1902) states that the chief criterion for the genus *Pelomyxa* is the presence of symbiotic bacteria in the cytoplasm. He says, "S'il me fallait donc caractériser le genre *Pelomyxa*, je le ferais à peu près en ces termes: 'Amibes à mouvements lents, toujours pourvues de bactéries symbiotiques.'" He then points out that Wilson's rhizopod has in common with the *Pelomyxa* only the *Glanzkörper* and that this form has nothing to distinguish it from the genus *Amoeba*.

Schaeffer (1918) in describing *Pelomyxa lentissima* states, "Other inclusions in the ectoplasm are the bacterial rods, distinctive of the genus." Later in his account of *P. schiedti* he says, "The bacterial rods, the presence of which characterizes the genus *Pelomyxa*, are found in considerable numbers in *schiedti*." Bourne (1891) in speaking of *P. viridis* describes the organism as, "densely packed with bacteria."

Thus it is seen that the chief characteristic of the genus *Pelomyxa* is the presence of symbiotic bacteria in the cytoplasm. Since the giant amoeba does not possess these bacteria, it is not a *Pelomyxa*.

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² Personal communication from Dr. B. D. Reynolds.

Schaeffer (1926, 1938a) maintains that Wilson's amoeba represents Roesel's (1755) "der kleine Proteus," and should therefore be called *Chaos chaos* Linnaeus. But Roesel's description is entirely inadequate to establish a species or even a genus. He undoubtedly had an amoeboid form, but it is impossible to find out the exact structure of his "Proteus." He mentions granules (*Körnern*) but says nothing of vacuoles, which in the giant amoeba are much larger than the crystals (granules). Schaeffer seems to base his conclusion mainly on the size and shape of the "Proteus." He contends that Roesel was a reliable investigator and that his figures may therefore be credited with general accuracy. As Schaeffer (1926) points out, "Roesel states the natural size of his amoeba in the rounded (spherical?) form to have been the same as figure A, which measures about 1660 μ in diameter." Yet Schaeffer (1938a) says, "*Chaos chaos* has a distinctive size range which fluctuates around 500 μ diameter." Thus, Schaeffer himself gives the diameter of the giant amoeba as about $\frac{1}{3}$ that of Roesel's "Proteus." There are also striking differences between Roesel's figures and description of a binary fission in his "Proteus" and Schaeffer's (1938b) photographs and account of the "3-daughter division of the giant amoeba."

Mast and Johnson (1931) review the data presented by Roesel and express the opinion that the latter possibly was dealing with a myxomycete. Rice (1945) states, "It is impossible to ascertain the exact structure of Roesel's 'der kleine Proteus.'" Thus since both the generic and specific names, *Chaos chaos*, are based on Roesel's inadequate description, they are not valid.

Hegner and Taliaferro (1924) in referring to Wilson's large amoeba use the name *Amoeba carolinensis* for it, though they give no reason for doing so. The present investigation adds evidence to support the use of this name. Therefore, *Amoeba carolinensis* (Wilson, 1900) Hegner and Taliaferro, 1924 is considered to be the correct name of the organism used in these experiments.

CYTOLOGY AND PHYSIOLOGY

MATERIALS AND METHODS

The original stock culture of *Amoeba carolinensis* was procured from the General Biological Supply House, Chicago, Illinois, in November 1944. About every two weeks subcultures were made in the following manner: Spring water containing five wheat grains per 100 cc. of water was boiled for a few minutes and left uncovered for several days. One or two of the grains of wheat were put into a butter dish and about 90 cc. of the boiled water was poured in, giving a depth of $\frac{3}{4}$ inches. The amoebae along with food organisms, which consisted chiefly of rotifers, *Chilomonas paramecium*, *Colpidium colpoda*, and *Paramecium caudatum*, were then added. In the best cultures a water mold grew on the wheat grains and many amoebae often were found among the hyphae of the mold. These stock cultures had been maintained for a little more than four months when the experiments began.

In addition to the wheat culture medium (W. C. M.) a hay medium (H. C. M.) was made as follows: 10 grams of chopped timothy hay were added to 1000 cc. of spring water and boiled for about 15 minutes. The solution was filtered to remove the solid particles and then put into test tubes with cotton stoppers and autoclaved on two successive days at 10 pounds pressure. From these test tubes the medium

was taken to start new cultures during the experiments. After trying various concentrations of this hay medium it was found that the amoebae and food organisms remained healthier and increased more rapidly if the stock solution was diluted with four parts of boiled and aerated spring water.

Cultures of *Chilomonas paramecium* and *Paramecium caudatum* were established in both the wheat and the hay culture media to serve as food organisms for the amoebae during the experiments. Subcultures were made from these about every two weeks.

Forty specimens of *Amoeba carolinensis* were washed six times in boiled spring water. Ten were transferred to two stender dishes (five to each dish) containing paramecia in W. C. M. In a similar manner ten were transferred to two stender dishes containing chilomonads in W. C. M.; ten, to two stender dishes containing paramecia in H. C. M.; and ten, to two stender dishes containing chilomonads in H. C. M. Each day the cultures were inspected and the supply of food organisms was replenished if it was low. The amoebae were counted daily for seven-day periods and then on the seventh day (in some cases a few days later) new cultures were made from the ones of the preceding week, so that by the end of the experiment most of the amoebae had been growing in one kind of medium and feeding on one kind of food organism for at least 46 days. With the exception of two cultures, the pH was taken on each culture on or after the seventh day. During the experiment, observations as to the relative size and form of the amoebae were made; some of the amoebae in each culture were examined in hanging drops under the compound microscope; and at the end of the experiment photomicrographs were taken of a few amoebae in the wheat-culture medium.

RESULTS

After the first two weeks there were marked differences in the rates of reproduction, especially between those amoebae grown in W. C. M. and fed on paramecia and the other cultures, as can be seen in Table I. During the first two weeks there seems to be a period of adjustment when the reproduction rates of the different cultures do not vary significantly. However, during the third to sixth weeks the amoebae grown in W. C. M. and fed on paramecia increased rapidly—from 10 to 45 during the sixth week. The other cultures showed little or no growth.

The hydrogen-ion concentrations remained rather constant. The pH's varied from 7.0 to 8.1, with that of the H. C. M. containing paramecia usually a little higher than the others. The room temperature varied from about 20° to 26° C.

There was no significant difference in size of the amoebae in the various cultures, but there was a difference in form. Those growing in W. C. M. and fed on paramecia were often somewhat disc-shaped with short, blunt pseudopods radiating from all sides as shown in Figure 1a. Others were usually monopodal or bipodal with blunt pseudopods as shown in Figure 1b. Clumping of the disc-shaped amoeba was observed five or six times.

The amoebae in W. C. M. fed on chilomonads were more flattened, with thinner pseudopods which were often at right angles to the main part of the body and frequently branched (Fig. 1c). The pseudopods often had little knob-like swellings at their distal ends (Fig. 1d). The amoebae in these cultures occasionally were monopodal in form.

TABLE I

Effect of culture media and food organisms on the reproduction rates of *Amoeba carolinensis*. At the beginning of each week amoebae were put into new cultures corresponding to the conditions under which they previously had been growing, so that the end results represent cumulative effects. Since there always were two cultures of each type, the pH's of both cultures are given.

	Wheat medium with paramecia				Wheat medium with chilomonads				Hay medium with paramecia				Hay medium with chilomonads			
	Number of amoebae		pH	% in- crease	Number of amoebae		pH	% in- crease	Number of amoebae		pH	% in- crease	Number of amoebae		pH	% in- crease
	First day	Sev- enth day			First day	Sev- enth day			First day	Sixth day			First day	Sev- enth day		
First week	10	17	7.5 7.6	70%	10	22	7.5 7.5	120%	10	18	7.7 7.8	80%	10	11	7.5 7.5	10%
Second week	10	15	7.6 7.6	50%	9	14	7.5 7.6	55½%	10	11	8.0 7.9	10%	10	10	7.8 7.7	0%
Third week	10	30		200%	10	10	7.8 7.9	0%	10	16	8.0 8.1	60%	10	10	8.0 8.1	0%
Fourth week	10	48	7.0 7.1	380%	10	10	7.6 7.6	0%	10	10	7.6 7.4	0%	10	11	7.9 8.0	10%
Fifth week	10	57	7.5 7.4	470%	10	13	7.6 7.6	30%	10	5	7.6 7.4	-50%	10	10	7.6 7.6	0%
Sixth week	10	45	7.3 7.4	350%	10	12	7.4 7.4	20%	5	4	7.8 7.6	-20%	10	10	7.3 7.2	0%

In H. C. M. the animals differed very little from each other as far as external appearance is concerned. They were, for the most part, monopodal or bipodal. Sometimes, however, those with the chilomonad diet exhibited the branched form characteristic of the amoebae in W. C. M. with chilomonads. Those amoebae with the paramecium diet were usually slightly larger than the ones feeding on chilomonads, and during the last two weeks one specimen grew to be the largest amoeba in any of the cultures. Very large vacuoles were apparent in the amoebae in H. C. M. with the paramecium diet, and some individuals were spherical. These last two characteristics are probably associated with degeneration.

In Wilson's description (1900) of this giant amoeba, he mentions minute, elongate, and fusiform crystals in the endoplasm. Wilber (1942) describes the crystals in more detail and says that there are two types: (1) plate-like crystals, and (2) bipyramidal crystals, which are the more numerous. He states that these crystals (presumably both kinds) are formed from food in the food vacuoles. Wilson also mentions spherical bodies 8μ in diameter and smaller, which resemble oil drops. Wilber gives the size range of these bodies as 2.5 – 8μ in diameter, and

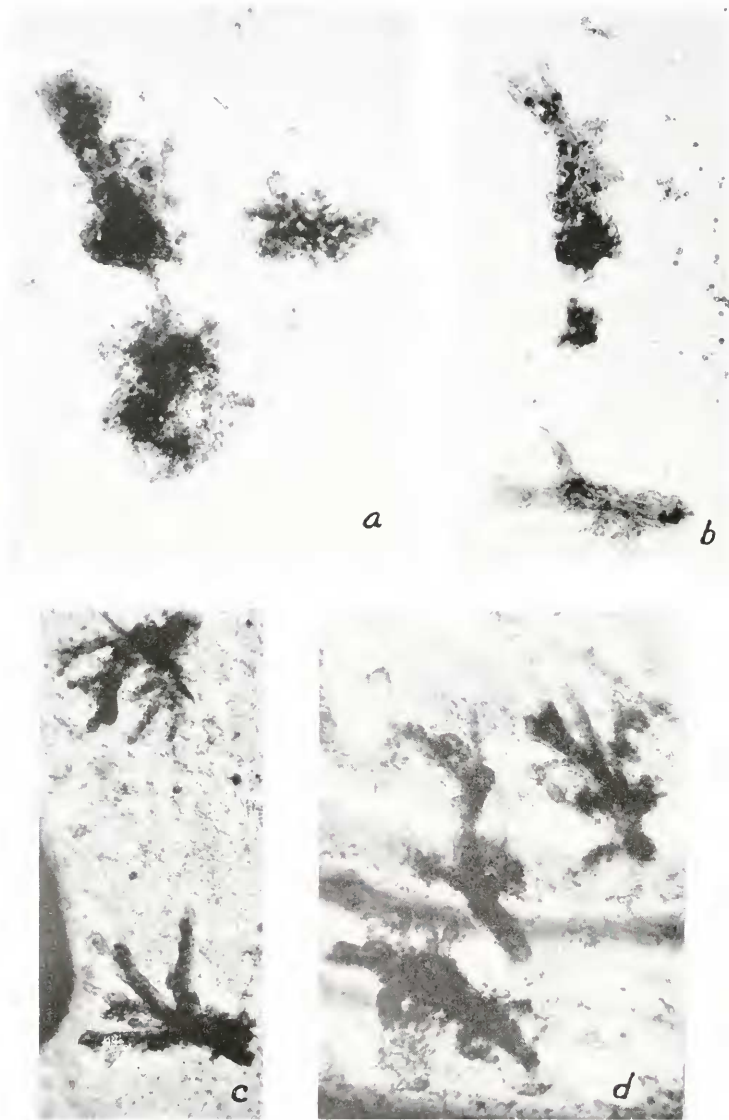


FIGURE 1. Photomicrographs of amoebae grown in wheat culture medium. *a* and *b*, those with paramecia as food organisms. *c* and *d*, those with chilomonads as food organisms.

concludes (1945) that they are formed from the crystals and the vacuole refractive bodies. He then says that the refractive spherical bodies function as reserve food in the amoeba.

While examining the stock and experimental amoebae under the compound microscope, differences in regard to the bipyramidal crystals and spherical bodies were noted. The plate-like crystals were so few in number that it was difficult to make a valid comparison in regard to their relative numbers and sizes.

In the stock cultures the amoebæ had few spherical bodies, the largest of which were about $5\ \mu$ in diameter. The largest bipyramidal crystals were about $2.4\ \mu$ long.

The amoebæ grown in W. C. M. with a diet of paramecia showed some larger bipyramidal crystals $2.8\ \mu$ long and a great number of smaller ones. There were few spherical bodies and the largest were only $3.1\ \mu$ in diameter.

Those specimens grown in W. C. M. and fed on chilomonads showed practically the same condition in regard to the bipyramidal crystals. However, there were very many large spherical bodies measuring $8.5\ \mu$ in diameter.

The spherical bodies of the amoebæ grown in H. C. M. and fed paramecia were slightly more numerous and a little larger than the ones in the amoebæ grown in W. C. M. with a paramecium diet. There were a few larger bipyramidal crystals $4.2\ \mu$ long, more $2.8\ \mu$ long and some smaller ones.

The amoebæ grown in H. C. M. with chilomonads showed many large bipyramidal crystals $4.2\ \mu$ long, and the spherical bodies were very large and numerous. Many of the latter were $9.8\ \mu$ in diameter, and some were slightly flattened on one side so that they looked like a three-quarter-full moon.

DISCUSSION

With the exception of size and number of nuclei, *Amoeba proteus* and *A. carolinensis* are very much alike. Schaeffer (1926) states, "My study of this matter (a comparison of *A. proteus* and *A. carolinensis*) leads me to include *diffusus* (*proteus*) in the same genus with *Chaos* (the giant amoeba). These two species, as a matter of fact, resemble each other more closely than most other species within one genus."

From Wilber's (1942) description of the cytology of *A. carolinensis* it is seen that the giant amoeba resembles *A. proteus* very closely in regard to the shape and structure of the nuclei and the cytoplasmic inclusions. Wilber (1945) has also shown that these two forms are very much alike in respect to the function of the nuclei, the formation of the contractile vacuoles, and the formation and function of the spherical bodies.

When the data of these experiments are compared with similar work on *A. proteus*, it is shown that these two amoebæ resemble each other in nutritional requirements. The numbers and relative sizes of the crystals and spherical bodies in the amoebæ in my experiments agree in general with the results of similar studies by Mast (1939) and Mast and Hahnert (1935) on *A. proteus*. Concerning the numerous spherical bodies in the chilomonad-fed amoebæ, Mast and Hahnert say, "This is of considerable importance for, as stated above, it indicates that the neutral red staining droplets found in abundance in *Chilomonas* but not in *Colpidium* function in the formation of the spherical bodies in *Amoeba*." My experiments indicate that this is also the case in *A. carolinensis*.

In examining the data of these nutrition studies it is seen that in general the amoebæ with the most numerous and largest crystals and spherical bodies had the lowest reproduction rates. In view of the fact that the spherical bodies serve as reserve food (Wilber, 1945), these experiments indicate that even though chilomonads may be ingested and digested by the amoebæ, this diet is not adequate for normal reproduction. The reproduction rates in the different cultures agree with the results of Reynolds (1938) and Williamson (1944) for *A. carolinensis*, but do not correspond with Williamson's data on *A. proteus*.

The relative sizes of the amoebae in the various cultures do not agree very closely with the observations during similar experiments by Mast (1939) on *A. proteus* and Williamson (1944) on *A. proteus* and *A. carolinensis*. In my experiments there was no increase in the size of the amoebae which had eaten ciliates, as found by both these authors, nor did the chilomonad-fed amoebae decrease in size, as found by Williamson.

In regard to the form of *A. carolinensis* while utilizing paramecia as food organisms, the results are in accord with those of Mast and Root (1916) for *A. proteus* and Williamson (1944) for *A. proteus* and *A. carolinensis*.

NUCLEAR DIVISION

MATERIALS AND METHODS

Specimens were taken from the stock cultures and fixed with Carnoy's, Belling's, and Bouin's fixatives. Those fixed with Carnoy's and Belling's fluids were stained with Delafield's and Heidenhain's haematoxylin. Those fixed in Bouin's fixative were stained with Heidenhain's haematoxylin. Some specimens were sectioned at 10μ . Since those fixed with Carnoy's fixative and stained with Heidenhain's haematoxylin showed the nuclear structure best and stained the cytoplasmic inclusions very little, all the remaining work on nuclear divisions was done with these reagents.

In order to secure nuclear divisions, slides were made at various hours of the day. All the specimens showing nuclear division stages were fixed between 5:45 p.m. and 11:00 p.m. and were more or less spherical in form. The experiments are not extensive enough to warrant any statement as to a periodicity of mitosis. This seeming periodicity was probably a result of better selection toward the end of the experiment.

OBSERVATIONS AND DESCRIPTIONS

Resting nucleus

The resting nucleus of *A. carolinensis* (Fig. 2a) is disc-shaped, and measures about $24 \times 10\mu$. Immediately beneath a distinct nuclear membrane and adhering closely to it are darkly staining granules (peripheral granules), the largest of which are about 2μ in diameter. The interior of the nucleus has a finely granular appearance and stains more lightly than the peripheral granules.

Schaeffer (1938) states that all the nuclei of an individual divide at the same time. Yet, nuclei at slightly different stages of division can be found in the same animal.

Prophase

In early prophase the nucleus enlarges apparently by a pulling away of the membrane from the central granules leaving a large endosome about 19μ in greatest diameter. The nucleus now measures $27 \times 15.4\mu$. Most of the peripheral granules are still rather small and stain more darkly than the endosome. Some of them however are slightly larger and less deeply stained than those in the resting nucleus. Faint strands running from the endosome to the periphery are now apparent.

In a little later stage (Fig. 2b and c) the nucleus has become more spherical measuring about $27 \times 22\mu$. Some of the peripheral granules have become loosened

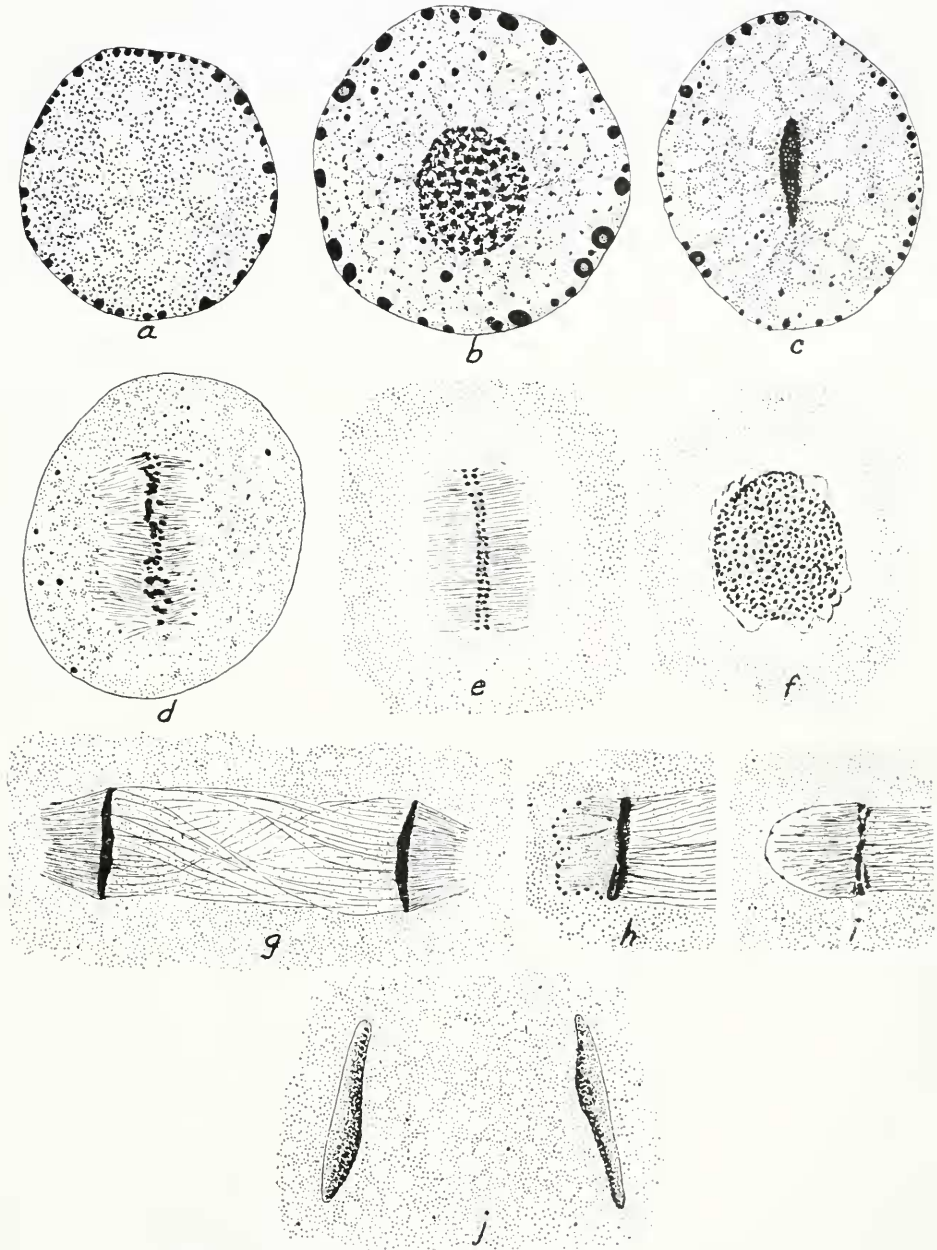


FIGURE 2. Camera lucida drawings of the stages of mitosis in *Amoeba carolinensis*. a, resting nucleus; b, prophase, face view; c, prophase, edge view; d, very late prophase showing chromosomes and spindle forming; e, metaphase, edge view; f, metaphase, polar view; g, late anaphase; h, early telophase, showing one plate with granules at the pole; i, a little later stage showing one pole with a delicate membrane and granules; j, late telophase with membrane fully formed. All drawings $\times 1660$.

from the membrane, are more spherical, and show a lighter area in the center. The endosome has become smaller and more compact, and now stains about as deeply as the peripheral granules. At this stage the endosome is a thin disc measuring $9.3\ \mu$ in diameter and $1.5\ \mu$ in thickness. The reticulum connecting the endosome with the periphery is more evident now.

Very late prophase (Fig. 2d) shows the chromosomes becoming arranged on the plate which is $14.2\ \mu$ in diameter. Spindle fibers are distinct and the peripheral granules have practically disappeared. A lighter area in the cytoplasm immediately surrounding the nucleus is visible. The nucleus shown in Figure 2d was the only nucleus in the animal in this condition. The remaining nuclei were in metaphase or early anaphase.

Metaphase

At metaphase (Fig. 2e and f) the nuclear membrane has disappeared. There are, however, around the periphery of the plate delicate, blister-like structures which may be remnants of the nuclear membrane. The chromosomes, which are spherical or ellipsoidal, and perhaps about 300 in number, are arranged on a discoidal plate $13.2\ \mu$ in diameter. The split halves of the chromosomes in some cases can be seen. The spindle fibers are at right angles to the chromosome plate and are about $4\ \mu$ long. No centrioles or granules are apparent at the ends of the spindle fibers. There is still a lighter area in the cytoplasm around the figure.

Anaphase

During anaphase (Fig. 2g) the chromosome plate splits into two daughter plates which diminish in diameter with the chromosomes becoming so closely aggregated that they no longer can be distinguished individually. In late anaphase the plates measure $8.4\ \mu$ in diameter and are, for the most part, flat. A few are slightly arched or saucer-shaped. The spindle between the chromosome groups has in most cases become twisted as if both plates had rotated in opposite directions. The polar fibers seem to be finer and more numerous than the interzonal ones, and the polar areas appear slightly darker than the surrounding cytoplasm. The polar fibers have not shortened during anaphase but are in most cases $5\ \mu$ long, and the outside ones are inclined at an angle of about 60° to the plates.

In an amoeba containing thirty-three nuclei, twenty-nine are in late anaphase and four are in early telophase. The nuclei in anaphase have their polar fibers inclined at an angle of 60° to the chromosome plates. The four in early telophase have granules arranged on a more or less hemispherical surface or membrane (Fig. 2h and i). Twenty-nine of the spindles lie so that the distance between daughter chromosome groups can readily be measured. The average distance between groups is $34\ \mu$. The shortest distance is $10\ \mu$, and the longest distance is $62\ \mu$. Some of the chromosome plates are tilted at angles to each other so that one of a pair is seen in edge view, while the other is seen in polar view. Some pairs of plates, both showing in edge view, are twisted at angles of 30° to 150° to each other. In the twenty-one anaphase spindles which lend themselves to analysis, twenty have the spindle between the chromosome groups twisted clockwise, or to the apparent right (Fig. 2g). One spindle shows no twisting. This constancy in direction indicates that the twisting is caused not by external forces in the cytoplasm, but by forces inherent in the spindle apparatus.

Telophase

In early telophase (Fig. 2h) granules appear at the distal ends of the polar fibers. A delicate, more or less hemispherical, membrane forms at the poles and the ends of the fibers nearest the poles disappear (Fig. 2i).

Later telophase (Fig. 2j) shows the plates larger in diameter, less dense and more finely granular, with the membrane more flattened. The granules are more densely packed on the median sides of the nuclei where the membrane is scarcely visible except near the edge of the disc. The spindle fibers have completely disappeared by this time.

DISCUSSION

Mitosis in *A. carolinensis* as herein described is very similar to that of *A. proteus* as described by Chalkley and Daniel (1933), Chalkley (1936), and Liesche (1938). The figures and descriptions of the nuclear division stages of *A. proteus* agree with those of *A. carolinensis* with the following exceptions:

1. The nuclei and mitotic figures of *A. carolinensis* are approximately half the size of those of *A. proteus*. Also the chromosome number of *A. carolinensis* (probably near 300) seems to be about half that given by Liesche (500–600) for *A. proteus*.

2. No "spireme" (Liesche) stage was observed in *A. carolinensis*.

3. No granules (Chalkley and Daniel) were visible at the distal extremities of the spindle fibers at metaphase.

4. During metaphase and anaphase grouping of the distal ends of the polar spindle fibers is not so pronounced in *A. carolinensis*, and during anaphase the chromosome plates are only slightly arched.

SUMMARY

1. It is concluded that the giant amoeba described by Wilson (1900) is not a *Pelomyxa* but belongs to the genus *Amoeba* and should be designated *A. carolinensis*. *Chaos chaos* is considered to be invalid as a name, owing to the fact that it is based on Roesel's inadequate description.

2. Nutritional studies indicate that *A. carolinensis* has food requirements similar to those of *A. proteus* and that these two species react in a similar manner to the same type of food.

3. Nuclear division is described for *A. carolinensis*. The stages are similar to those described for *A. proteus*, and except for smaller size, correlated with a smaller number of chromosomes, the differences are insignificant.

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